2015 Cambridge - JIC

Microscopy Workshop

Objectives

1. Gain experience using different microscopes and develop an understanding of what can be achieved with each one.
2. Get the chance to make up some slides for imaging.
3. Image some interesting samples from the zoology and geology departments.
4. Gain first hand experience of how microscopy is being used within the field of synthetic biology.

Safety Requirements

1. Ensure to wash hands before and after carrying out the lab.
2. No food or drink is to be brought into the lab.
3. Wear lab coats throughout the workshop.
4. Wear gloves when carrying out any tasks in the wet lab
5. Gloves are not to be worn when using the microscopes
6. Ensure to dispose of items and liquids properly as instructed by the lab technician.

Activity 1: Preparing Cheek Cell Slides

Cheek cells can be scraped off the outer epithelial layer of the mouth when a swab is taken. These are squamous epithelial cells. Under the microscope it should be possible to see their cell membranes and nuclei.

Apparatus

- Slides
- Cover slips
- Pipettes
- Sterile, individually packed cotton swabs
- Distilled water

Method

1. Take a clean swap and scrape the inside of the cheek gently.
2. Smear the cotton swap on the center of the microscope slides.
3. Using a pipette add a drop of water to the slide.
4. Place a coverslip carefully over the sample: this can be done by holding it an angle using the pipette or tweezers and lowering it slowly onto the slide.

Activity 2: Preparing GFP Marchantia Slides

The tool used in this activity to prepare thin Marchantia samples is the microtome. If good technique is carried out in the preparation it should be possible to slice the Marchantia so thin that it is possible to see a single layer of plant cells under the microscope. These Marchantia contain GFP (Green Fluorescent Protein). This causes the cell membranes of the cells to glow green when they are excited under the fluorescence microscope.

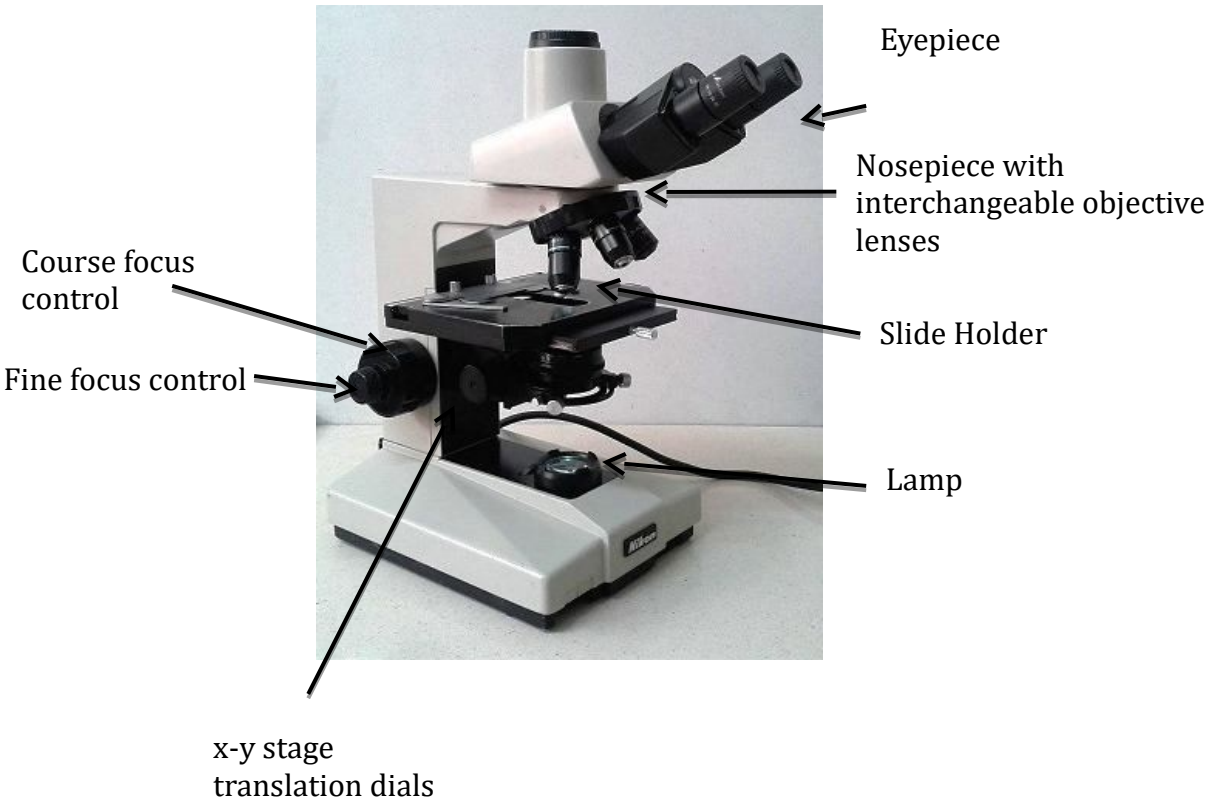
Apparatus

- Slides
- Cover slips
- Pipettes
- Tweezers
- Knife
- Chopping board
- Carrot samples soaked in 70% alcohol
- Microtome
- Distilled water

Method

1. Remove a tubular carrot from the alcohol using the tweezers.
2. On the chopping board cut the carrot in half along the middle.
3. Remove a small flat piece of the *Marchantia*, using the tweezers
4. Sandwich the flat piece between the two carrot halves, at the top.
5. Then sandwich the carrot in between the two pieces of cork and place within the microtome holder.
6. Place the holder within the microtome. Rotate the microtome until the top of the carrot is just below the surface.
7. Spray distilled water onto the top on the microtome
8. Rotate the carrot up while at the same time using the knife to slice thin pieces of carrot and *Marchantia*.
9. Using the water wash these off of the knife and into a beaker.
10. Use a pipette to remove one drop of water containing the small sample of *Marchantia* and place it on the slide. This can be repeated several times if it is wished for several samples in the one go.
11. Place a coverslip carefully over the sample: this can be done by holding it an angle using the pipette or tweezers and lowering it slowly onto the slide.
12. Use some tissue to dry up any excess water.

Activity 3: Imaging your Samples



1. Turn on the microscope and the lamp.
2. Clip the slide into the slide holder
3. Turn the nosepiece to the 10x objective lens
4. Using the course focus move the stage until it is 1mm beneath the objective lens (care should be taken so ensure that the objective lens and the stage do not come into contact, possibly damaging the lens!).
5. Move the stage down until the sample comes into focus.
6. Rotate the nosepiece to the required objective lens and refocus using course and fine adjustment.
7. The sample can be moved around on the stage using the x-y stage translation dials.
8. There will be the chance to image on the lab's fluorescence microscope: one of the team will provide further direction on how to use this.

Activity 4: Imaging Drosophila Flies from the Zoology Department

This activity will involve the fruit fly *Drosophila Melanogaster.* It is a 'model organism' in the field of biology, it is one of the main organisms on which biological research is carried out.

What areas of research are *Drosophila* used in?

- Developmental biology: looking at how one cell makes a whole organism
- Genetics: looking at how genes are controlled
- Neurobiology: looking at how nerves and brains work

Why are they used?

- They're multicellular animals, like us
- *Drosophila* development and genomics are well understood
- The tools to study them are well developed
- They're easy to keep and have a short generation time (so fast results)
- They have a nervous system similar to humans that we can study
- Many features evolutionarily conserved in humans
- They're much easier to study than humans (for ethical and practical reasons)

In this lab try to recognise certain features of the *Drosophila*: their eyes, embryos, legs and wings.

Activity 5: Imaging Geological Samples Under a Petrographic Microscope

The petrographic microscope is a special type of microscope used to look at crystals and minerals. It has 2 polarising filters that give very interesting effects. Light is polarised in one direction and then allowed to travel through the crystal/rock samples. Some of the light travelling through the sample is rotated and slowed down a bit, which gives rise to strange colours in some crystals. This, along with a few other features of the microscope, allows identification of sample composition and helps you distinguish how samples were created.